Immunosuppressive Macrolides of the Type FK 506: A Novel Class of Topical Agents for Treatment of Skin Diseases?

Josef G. Meingassner and Anton Stütz
Department of Dermatology, Sandoz Forschungsinstitut, Vienna, Austria

The immunosuppressive macrolide antibiotics FK 506 and rapamycin were tested for topical activity in experimental allergic contact dermatitis of farm pigs. This species was used because pig skin, in comparison to rodent skin, resembles human skin more closely. For comparison, cyclosporine A (CyA), which is orally but not topically active in patients with skin disease, dexamethasone, and clobetasol propionate were used. Treatment was performed twice, 30 min and 6 h after elicitation of challenge reaction. Topical application of 0.4 to 0.04% FK 506 caused a pronounced inhibition of inflammatory skin reactions of hypersensitivity to dinitrofluorobenzene. The treatment response was similar to the activity of 0.13% clobetasole. Dexamethasone (1.2%) was less active than clobetasol. In contrast, rapamycin and CyA were inactive at concentrations of 1.2 and 10%, respectively. Because the pig data on corticosteroids and cyclosporine A are in agreement with clinical findings, these studies indicate that immunosuppressive macrolides of the type FK 506 may be useful drugs for the topical treatment of human skin diseases that respond to local corticosteroids and oral treatment with cyclosporine A. *J Invest Dermatol 98:851–855, 1992*

Until recently, the common armamentarium for systemic or topical therapy of inflammatory and hyperproliferative dermatoses was limited to regimens such as corticosteroids, retinoids, methotrexate, psoralen plus ultraviolet A light, ultraviolet B, coal tars, or anthralin. Cyclosporine A (CyA), which was first used for the prevention of rejection of transplanted organs, has become a valuable additional drug for systemic treatment of psoriasis [1,2]. Its therapeutic value in other skin disorders such as atopic dermatitis, lichen planus, and pyoderma gangrenosum is being currently evaluated [3–6]. The extent of CyA's future use in the systemic treatment of such skin disorders will be determined by the risk-benefit ratio associated with its side effects. Topical CyA application, which would minimize such side effects, has not been therapeutically successful: in psoriasis, allergic contact dermatitis (ACD), atopic dermatitis, and alopecia areata the response to topical CyA is at best marginal [7–14]. The lack of topical efficacy seems to be due to insufficient skin penetration [15], because intralesional injections caused significant improvement of chronic plaque psoriasis [16,17] and patients with oral lichen planus responded well to topical CyA (mouthwash therapy) [18–20]. In contrast to epicutaneous treatment of patients in clinical trials, topical application of CyA was effective in a variety of skin models in small laboratory animals [21–25]. In particular, ACD showed a dose-dependent response in mice and guinea pigs, whereas no response or minimal response was observed in humans.

In search for an animal model that resembles more closely the situation in clinical studies, we have developed a pig model for ACD. The choice of pigs was based upon the permeability properties of pig skin, which is known to more closely resemble human skin [26] than rodent skin.

Recently, the macrolide antibiotics FK 506 and rapamycin have been reported to be potent immunosuppressive drugs [27–32,37–39]. Structurally unrelated to the cyclic peptide CyA (Fig 1), both FK 506 and rapamycin inhibit T-cell activation but at 10–100 times lower concentrations than CyA [27–30,37,38]. On the other hand, distinct mechanisms of suppression of T-cell proliferation have been reported for FK 506 and rapamycin despite their striking structural similarity [35,36,40]. Assuming that anti-T-cell activities of CyA are responsible for, or at least contribute to, its topical efficacy in murine ACD as well as for its systemic efficacy in human skin diseases, it was of interest to investigate the topical activity of FK 506 and rapamycin in ACD of pigs. This study was performed in comparison with the corticosteroids dexamethasone and clobetasol as well as with CyA.

MATERIALS AND METHODS

Groups of four to six female domestic pigs (Landrasse, weighing approximately 12 kg) were sensitized with 10% dinitrofluorobenze [(DNFB), solution in 50% acetone, 10% dimethylsulfoxide (DMSO) and 30% olive oil] applied in volumes of 100 μl onto both auricles (medial aspects) and groins on day 1. The animals were exposed again on the lateral aspects of both auricles to 100 μl 2% DNFB on day 4. Challenge was performed with a 1% DNFB solution (without DMSO) on day 12 by applying 20 μl epicutaneously to each of 24 test sites (2 cm diameter) arranged in four craniocaudal lines on the dorsolateral back of each animal. The test sites were treated twice (0.5 and 6 h after challenge) with 20 μl solution of active compound or drug vehicle. FK 506, rapamycin (both compounds obtained from the Department of Biotechnology, Sandoz

Manuscript received May 17, 1991; accepted for publication November 18, 1991.
Presented in part at the Annual Meeting of the Society for Investigative Dermatology, Seattle, Washington, May 1–3, 1991.
Reprint requests to: Dr. Meingassner, Sandoz Forschungsinstitut Wien, Brunner Strasse 59, A-1235 Vienna, Austria.
Abbreviations:
ACD: allergic contact dermatitis
CyA: cyclosporine A

0022-202X/92/$05.00

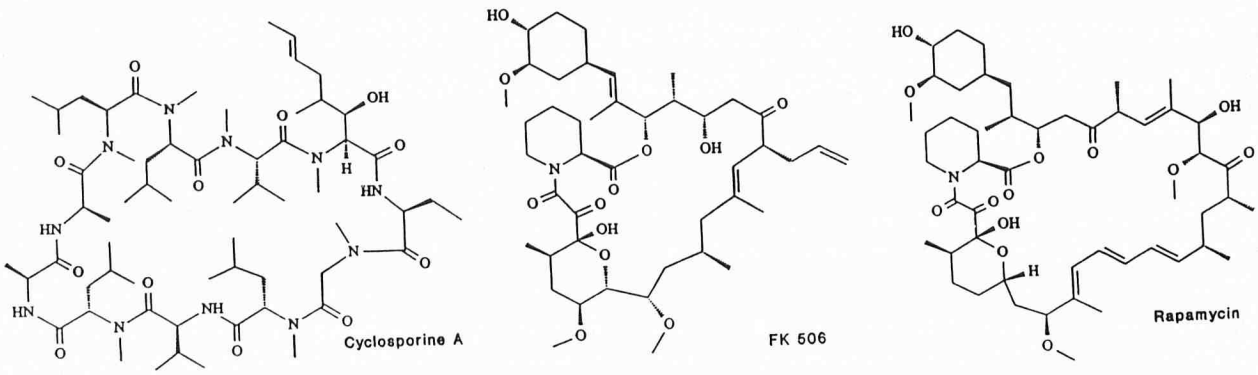

Figure 1. The structures of CyA, FK 506, and rapamycin.

Basle), CyA, and clobetasol-17-propionate were prepared with ethanol/propylene glycol (3:7). Dexamethasone was dissolved in dimethylacetamide/ethanol/propylene glycol (1:2:4). The corticosteroids were used as reference compounds. Efficacy of each preparation was determined on two pigs on a total of 12 test sites. Evaluation of the treatment-related effects was performed at the peak inflammatory response, which was 24 h after the challenge. Each test site was evaluated visually for (a) intensity, (b) extent of erythema, and (c) consistency. These three parameters were arbitrarily scored on a scale from 0 to 4, allowing a combined maximal score of 12 per designated site. For (a) intensity of erythema, the scores were as follows: 0, no erythema; 1, barely perceptible; 2, slight; 3, moderate; and 4, severe. For (b) extent of erythema the scores were 0, no erythema; 1, macules of pinhead size; 2, lentil-sized macules; 3, confluent macules; and 4, diffuse. For (c) consistency of lesion, the scores were 0, normal finding; 1, nodules of pinhead size; 2, doughy lentil-sized nodules; 3, confluent firm nodules; and 4, diffuse hard lesion. Clinical observations were performed in a blind fashion by the same examiner. In addition, skin changes were biophysically characterized by measuring microvascular perfusion (PeriFlux PF3 Laser Doppler Perfusion Monitor) and reflective color measurement (Minolta Chroma Meter CR 200). For quantitative readings perfusion units and a* values estimating changes in the redness intensity according to the L*a*b* system were used [33].

Statistics Blood flow units and a* values were evaluated by Student t test; when data did not conform to a normal distribution, these values were analyzed by Wilcoxon-Mann-Whitney U test, as were the clinical scores. Observed differences were considered significant if $p \leq 0.05$. The software used was the program system RS1, procedure Compare. The percentage inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{mean}^{+} \text{ of VS} - \text{mean of DS}}{\text{mean of VS}} \times 100,$$

where + is the median values for clinical scores, VS is the sites treated with the vehicle, and DS is the sites treated with the drug.

RESULTS

Topical application of FK 506 at concentrations ranging from 0.4 to 0.04% caused a pronounced inhibition of inflammatory skin reactions of hypersensitivity to DNFB in pigs (Tables I and II and Figs 2–4). Even at a concentration of 0.04%, depending on the parameter used, inhibition in the range of 40–56% was observed. A protracted inflammatory response in FK 506–treated animals was not noted when the test sites were read 48 h after the challenge.

In contrast, topical application of 1.2 and 0.13% rapamycin and 10% cyclosporine A proved to be inactive. Dexamethasone (1.2%) was moderately active with 25–38% inhibition, whereas 0.13% clobetasol inhibited inflammation by 37–76%, certifying the different anti-inflammatory potency of these corticosteroids also in the pig-skin model. A pronounced skin atrophy was observed visually at clobetasole-treated test sites a few days after application.

DISCUSSION

Taking into consideration the negative results obtained with CyA, the fact and degree of inhibition of inflammation by topical FK 506 in the pig model was quite unexpected, because the high molecular

Table I. Influence of Topical Treatment with Drug (DS) and Placebo Formulations (VS) on Skin Reddening of ACD-Test Sites in Domestic Pigs

Drug	Concentration (%)	DS	VS	Significance, DS versus VS, "p" Values	Efficacy of DS (% inhibition)
FK 506	0.40	2.2[a] ± 0.7	9.4 ± 2.0	≤0.001	77
FK 506	0.13	2.4 ± 0.6	7.6 ± 1.4	≤0.001	68
FK 506	0.04	3.8 ± 0.7	7.8 ± 2.6	≤0.05	51
Rapamycin	1.20	10.9 ± 1.6	10.9 ± 0.8	NS[b]	0
Rapamycin	0.13	10.3 ± 1.5	10.7 ± 1.0	NS	4
Cyclosporine A	10.00	3.3 ± 0.8	3.6 ± 1.0	NS	8
Dexamethasone	1.20	3.3 ± 1.4	5.3 ± 2.1	NS	38
Clobetasol	1.20	0.6 ± 1.7	4.5 ± 1.5	≤0.01	87
Clobetasol	0.13	1.1 ± 0.8	4.5 ± 2.0	≤0.01	76

[a] Skin color was measured by reflectometry as described in *Materials and Methods*, data (a* values of the L* a* b* system) are expressed as mean ± SD of 12 test sites.
[b] NS, not statistically significant.

Table II. Influence of Topical Treatment with Drug (DS) and Placebo Formulations (VS) on Microvascular Perfusion of ACD-Test Sites in Domestic Pigs

Drug	Concentration (%)	DS	VS	Significance, DS versus VS, "p" Values	Efficacy of DS (% inhibition)
FK 506	0.40	41 ± 10.1[a]	114 ± 15.0	≤0.001	64
FK 506	0.13	47 ± 12.3	95 ± 10.1	≤0.001	51
FK 506	0.04	55 ± 5.1	91 ± 2.1	≤0.001	40
Rapamycin	1.20	NT[b]	NT	—	—
Rapamycin	0.13	NT	NT	—	—
Cyclosporine A	10.00	66 ± 6.0	69 ± 5.0	NS[c]	4
Dexamethasone	1.20	63 ± 7.1	84 ± 19.4	≤0.05	25
Clobetasol	1.20	59 ± 20.7	105 ± 27.1	≤0.01	44
Clobetasol	0.13	77 ± 15.6	123 ± 10.4	≤0.001	37

[a] Blood flow units expressed as mean ± SD of 12 test sites.
[b] NT, not tested.
[c] NS, not statistically significant.

weight of FK 506 (MW 804) was anticipated to be a limiting factor for penetration as it is most likely the case with CyA (MW 1202). However, FK 506 was obviously sufficiently absorbed to be topically active. FK 506 proved to be clearly superior to dexamethasone. The activities of 0.04% FK 506 were even similar to a 0.13% formulation of the superpotent corticosteroid clobetasol-17-propionate, which, however, caused severe local side effects. Similar treatment-related skin lesions were not observed at FK 506-treated sites. Cutaneous atrophy is a significant side effect of topical corticosteroids and it appears that atrophogenicity correlates directly with potency [28]. Topical activity of FK 506 in ACD is probably more specific than that of corticosteroids and thus associated with less side effects.

In contrast to FK 506, the structurally related macrolide rapamycin turned out to be topically ineffective in the pig model, although both compounds have been reported to be immunosuppressants of comparable potency and their physicochemical properties seem to be similar also. In addition, and distinct from CyA, topical rapamycin did not inhibit contact hypersensitivity-induced pinnal swelling of mice (data not shown). These findings raise the question of the mechanisms involved in inflammation due to contact hypersensitivity and how FK 506 interferes with these processes in contrast with rapamycin. Similar to CyA, FK 506 has been reported to block T-cell proliferation primarily by interfering with the transcription of early lymphokines (e.g., IL-2, IL-4) without impairing their response to exogenous lymphokines. In contrast, rapamycin appears to inhibit T-cell proliferation at a later point in the activation process by impairing their response to growth lymphokines. CyA and FK 506 inhibit Concanavalin A-stimulated mouse spleen cells from entering the cell division cycle at the Go/G1 interface. Rapamycin acts later at some point in G1 [35,36,40]. These integral mechanistic differences are obviously associated with distinct gene control mechanisms resulting in various gene products. Abrogation of release of cell cycle-dependent "proinflammatory" lymphokines in CyA- and FK 506-treated animals might be the cause of therapeutic response in contrast to rapamycin-treated animals in which recruitment of inflammatory cells is not inhibited, which in turn cause the gradually intensifying eczematous reaction. Distinct

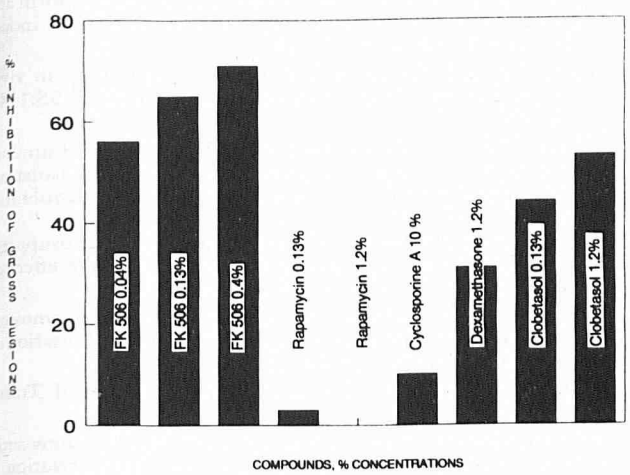

Figure 2. Efficacy of FK 506, rapamycin, clobetasol, dexamethasone, and cyclosporine A on ACD of domestic pigs, assessed by scores for clinical findings. Results are expressed as a percentage of inhibition of gross lesions, as described in *Materials and Methods.*

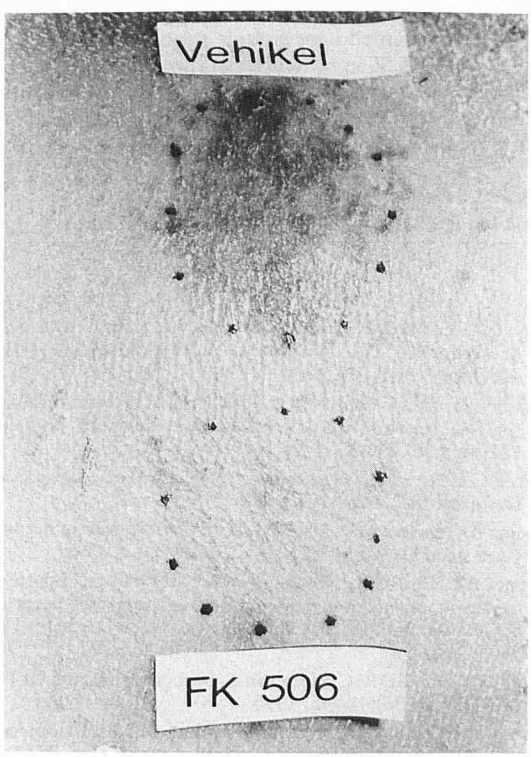

Figure 3. Gross changes of allergic contact dermatitis of test sites treated vehicle *(above)* and 0.13% FK 506 *(below)*.

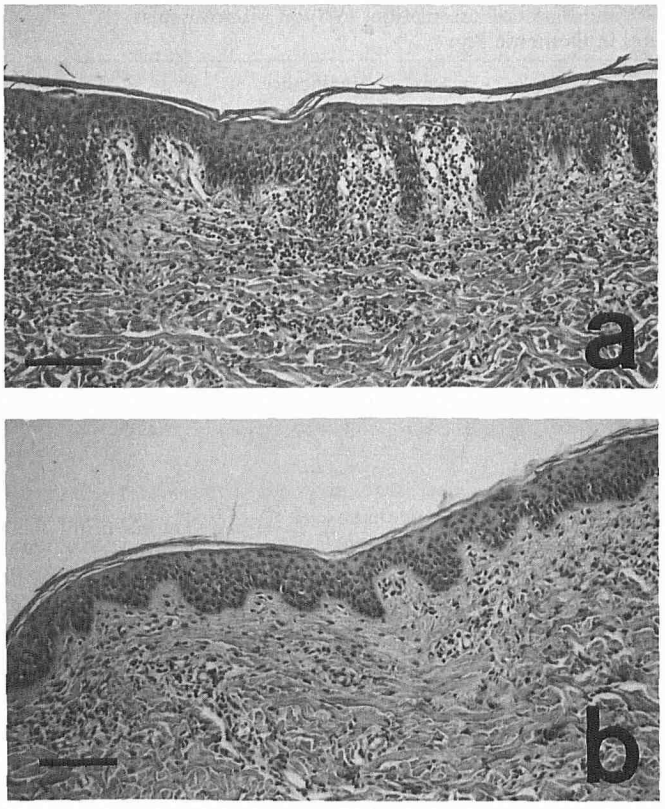

Figure 4. Microscopic changes of allergic contact dermatitis of test sites treated with vehicle *(a)* and with 0.13% FK 506 *(b)*. *Bar,* 100 μ.

effects of CyA and FK 506 on other cells (like Langerhans' cells, mast cells) have also to be considered.

The results presented here provide strong evidence that macrolides of the type FK 506 may represent a novel class of topical agents for the treatment of contact hypersensitivity and probably for other skin diseases responding to topical corticosteroids and to systemic CyA treatment.

We thank Mr. H. Fahrngruber for excellent technical assistance.

REFERENCES

1. Powles AV, Baker BS, Valdimarsson H, Hulme B, Frey L: Four years of experience with cyclosporin A for psoriasis. Br J Dermatol 122(supp 36):13–19, 1990
2. Fradin MS, Ellis CN, Voorhees JJ: Efficacy of cyclosporin A in psoriasis: a summary of the United States' experience. Br J Dermatol 122(supp 36):21–25, 1990
3. Biren CA, Barr RJ: Dermatologic applications of cyclosporine. Arch Dermatol 122:1028–1032, 1986
4. Gupta AK, Brown MD, Ellis CN, et al: Cyclosporine in dermatology. J Am Acad Dermatol 21:1245–1256, 1989
5. Gupta AK, Ellis CN, Nickoloff BJ, et al: Oral cyclosporine in the treatment of inflammatory and non-inflammatory dermatoses. A clinical and immunopathologic analysis. Arch Dermatol 126:339–350, 1990
6. Ross JS, Camp RDR: Cyclosporin A in atopic dermatitis. Br J Dermatol 122(supp 36):41–45, 1990
7. Coulson IH, Holden CA: Topical cyclosporin A in alopecia totalis: failure of therapeutic effect due to lack of penetration. Br J Dermatol 121(suppl 34):53–54, 1989
8. Parodi A, Rebora A: Topical cyclosporine in alopecia areata. Arch Dermatol 123:165–166, 1987
9. Aldridge RD, Sewell HF, King G, Thomson AW: Topical cyclosporin A in nickel contact hypersensitivity: results of a preliminary clinical and immunohistochemical investigation. Clin Exp Immunol 66:582–589, 1986
10. Gilhar G, Winterstein G, Golan DT: Topical cyclosporine in psoriasis. J Am Acad Dermatol 18:378–379, 1988
11. Prost Y de, Bodemer C, Teillac D: Double-blind randomized placebo-controlled trial of local cyclosporine in atopic dermatitis (letter). Arch Dermatol 125:570, 1989
12. Griffiths CEM, Powles AV, Baker BS, Fry L, Valdimarsson H: Topical cyclosporin and psoriasis (letter). Lancet I:806, 1987
13. Thomson AW, Aldridge RD, Sewell HF: Topical cyclosporin in alopecia areata and nickel contact dermatitis. Lancet II:971–972, 1986
14. Lembo G, Balato N, Patruno C, Ayala F: Topical application of cyclosporine on guinea pig allergic contact dermatitis. Arch Dermatol 125:1431–1432, 1989
15. Hermann RC, Taylor RS, Ellis CN, et al: Topical cyclosporin for psoriasis: in vitro skin penetration and clinical study. Skin Pharmacol 1:246–249, 1988
16. Powles AV, Baker BS, McFadden J, Rutman AJ, Griffiths CE, Frey L, Valdimarsson H: Intralesional injection of cyclosporin in psoriasis. Lancet I:537, 1988
17. Ho VC, Griffiths CE, Ellis CN, et al: Intralesional cyclosporine in the treatment of psoriasis. A clinical, immunologic, and pharmacokinetic study. J Am Acad Dermatol 22:94–100, 1990
18. Frances C, Boisnic S, Etienne S, Szpirglas H: Effect of the local application of cyclosporine A on chronic erosive lichen planus of the oral cavity. Dermatologica 177:194–195, 1988
19. Balato N, Rosa de S, Bordone F, Ayala F: Dermatological application of cyclosporine. Arch Dermatol 125:1430–1431, 1989
20. Eisen D, Ellis CN, Duell EA, Griffiths CE, Voorhees JJ: Effect of topical cyclosporine rinse on oral lichen planus. A double-blind analysis. New Engl J Med 323:290–294, 1990
21. Meingassner JG, Bavandi A, Petranyi G: Activity of Sandimmune (cyclosporine A) in dermatitis models (abstr). J Invest Dermatol 94:555, 1990
22. Aldridge RD, Thomson AW, Rankin R, Whiting PH, Cunningham C, Simpson JG: Inhibition of contact sensitivity reactions to DNFB by topical cyclosporin application in the guinea-pig. Clin Exp Immunol 59:23–28, 1985
23. Gschwendt M, Kittstein W, Marks F: Cyclosporin A inhibits phorbol ester-induced cellular proliferation and tumor promotion as well as phosphorylation of a 100-kd protein in mouse epidermis. Carcinogenesis 8:203–207, 1987
24. Fisher GJ, Gupta AK, Duell EA, Elder JT, Nickoloff BJ, Voorhees JJ: Cyclosporine A inhibits the TPA-induced inflammatory hyperplastic response in mouse skin (abstr). J Invest Dermatol 90:559, 1988
25. Griffiths RJ, Wood BE, Li S, Blackham A: Effects of cyclosporin and protein synthesis inhibitors on cutaneous inflammation in mouse skin. Skin Pharmacol 2:30–37, 1989
26. Barteck MJ, La Budde JA, Maibach HI: Skin permeability in vivo: comparison in rat, rabbit, pig and man. J Invest Dermatol 58:114–123, 1972
27. Kino T, Hatanaka H, Hashimoto M, et al: FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. J Antibiotics 40:1249–1255, 1987
28. Kino T, Hatanaka H, Miyata S, et al: FK 506, a novel immunosuppressant isolated from a Streptomyces: II. Immunosuppressive effect of FK 506 in vitro. J Antibiot 40:1256–1265, 1987
29. Ochiai T, Nakajima K, Nagata M, et al: Effect of a new immunosuppressive agent, FK 506, on heterotopic cardiac allotransplantation in the rat. Transplant Proc 19:1284–1286, 1987
30. Thomson AW: FK 506—how much potential? Immunol Today 10:6–9, 1989
31. Sehgal SN, Baker H, Vezina C: Rapamycin (AY-22,989), a new antifungal antibiotic: II. Fermentation, isolation and characterization. J Antibiot 28:727–732, 1975
32. Martel RR, Klicius J, Galet S: Inhibition of the immune response by rapamycin, a new antifungal antibiotic. Can J Physiol Pharmacol 55:48–51, 1977
33. Babulak SW, Rhein LD, Scala DD, Simion FA, Grove GL: Quantita-

tion of erythema in a soap chamber test using the Minolta Chroma (Reflectance) Meter: comparison of instrumental results with visual assessments. J Soc Cosmet Chem 37:475–479, 1986

34. Meyrick Thomas RH, Black MM: Corticosteroids: cutaneous atrophy. In: Maibach HI, Lowe NJ (eds.). Models in Dermatology Vol. 2. Karger, Basel, 1985, pp 30–14
35. Metcalfe SM, Richards FM: Cyclosporine, FK 506, and Rapamycin. Transplantation 49:798–802, 1990
36. Dumont FJ, Staruch MJ, Koprak SL, Melino MR, Sigal NH: Distinct mechanisms of suppression of murine T cell activation by the related macrolides FK-506 and rapamycin. J Immunol 144:251–258, 1990
37. Sawada S, Suzuki G, Kawase Y, Takaku F: Novel immunosuppressive agent, FK 506: in vitro effects on the cloned T cell activation. J Immunol 139:1797–1803, 1987
38. Yoshimura N, Matsui S, Hamashima T, Oka T: Effect of a new immunosuppressive agent, FK 506, on human lymphocyte responses in vitro. I. Inhibition of expression of alloantigen-activated suppressor cells as well as induction of alloreactivity. Transplantation 47:351–356, 1989
39. Yoshimura N, Marsui S, Hamashima T, Oka T: Effect of a new immunosuppressive agent, FK 506, on human lymphocyte response in vitro: II. Inhibition of the production of IL-2 and gamma INF, but not B cell-stimulating factor-2. Transplantation 47:356–359, 1989
40. Kimball PM, Kerman RH, Kahan BD: Rapamycin and cyclosporine produce synergistic but nonidentical mechanisms of immunosuppression. Transplant Proc 23:1027–1028, 1991